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Comparative Studies of Phytochemicals from the Leaf and Flower Extracts of *Piliostigma thonningii* and their Antibacterial Efficacy

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ABSTRACT:
This research explores and compares the phytochemical constituents of the leaf and flower extracts of *Piliostigma thonningii* and their possible antibacterial application. Employing gas chromatography-mass spectrometry technique, twenty-six phytochemical components were identified in the leaf comprising esters (47.23 %), fatty acids (38.87 %), alcohols (5.22 %), hydrocarbons (2.53 %), organic chloride (2.46 %), ketones (2.20 %), amide (0.93 %) and aromatic (0.56 %), while twenty-five were identified in the flower consisting fatty acids (52.42 %), hydrocarbons (32.74 %), terpene/terpenoids (4.75 %), ether (2.54 %), thiol (1.80 %), phenolic (1.78 %), halogenoalkane (1.52 %), aromatic alcohol (1.48 %) and ester (0.90 %). Using the disc diffusion method, both extracts inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*. However, the flower extract showed a more potent inhibitory effect against the two bacteria organisms. Gentamycin was used as a standard antibacterial agent and its sensitivity was comparable to those of the extracts. The ability of the extracts to show significant antibacterial activity against the tested organisms could be the reason why the plant is used in herbal medication for the treatment of diseases and infections owing to the presence of enormous bioactive phytochemicals in them.

Keywords: Phytochemicals, *Piliostigma thonningii*, GC/MS Analysis, Antibacterial, Herbal medication

INTRODUCTION

Phytochemicals are plant chemicals that possess bioprotective properties. They can function as antioxidants, antibacterials, immune-stimulants, and anti-inflammatory agents among other myriad biological importance [1]. Hence the need to explore the phytochemical components of the leaf and flower extracts of *Piliostigma thonningii*.

P. thonningii is a species of flowering plant that belongs to the family Fabaceae [2]. The plant is native to Africa. It is a deciduous or evergreen shrub or tree with a rounded crown and can grow 3 - 15 metres tall [3]. It flowers from December to February [4]. An interesting feature of the plant is that the male and female flowers occur on different trees in most cases. If on the same tree, male flowers occur first and then female flowers later so that self-pollination is not possible [4]. Flowers are not showy and are followed by large, thick, reddish brown, non-splitting pods about 30–70 mm long [4].

The leaf extract of *P. thonningii* has been reported to be used in herbal medication for the treatment of diarrhoea, dysentery, cough, worms and other intestinal problems [5]. A decoction of the leaf is drunk and bathed against fever, toothache, and epilepsy. It is also used as a vaginal wash and as an enema to a mother giving birth [6]. The leaf preparations are antiseptic and are used to promote wound healing, treat skin diseases and snake bites. The tender leaves are chewed and the juice swallowed to treat stomach-ache [4]. The powdered dried flowers are eaten in food, drunk in water or smoked like tobacco against a cough [6]. The fresh leaves and flowers of this tree can be chewed to reduce thirst. The bark infusions are used to treat diarrhoea. There is evidence that this plant is used in most African countries by traditional medicine practitioners for managing a variety of ailments like ulcers, gastric and heart pains and erectile dysfunction [6].

In view of the importance of *P. thonningii* in herbal medication, it becomes pertinent to investigate the phytochemical constituents of its leaf and flower extracts using GC/MS technique as well as to study their antibacterial potency.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh leaves and flowers of *P. thonningii* were collected from the tree plant located in front of the College of Physical and Applied Sciences, Michael Okpara University of Agriculture, Umudike, Abia State. The plant materials were identified and authenticated by a specialist in Plant Taxonomy of Taxonomy Section, Forestry Department of the same university.

Extraction of Phytochemicals

The fresh leaves and flowers of *P. thonningii* were washed thoroughly with running tap water and rinsed with distilled water to remove dust

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and impurities. The plant materials were shade-dried and ground into fine powder using a laboratory milling machine. The cold batch extraction method was employed using chloroform. In a typical extraction procedure, 20 g of the powdered sample was introduced into 500 ml volumetric flask and 200 ml of chloroform was added. The flask was covered with the aid of a filter paper and rubber-band and then stirred continuously in a shaker for 24 h. The mixture was then filtered through Whatman No. 1 filter paper and the filtrate was concentrated by allowing the chloroform to evaporate out at room temperature.

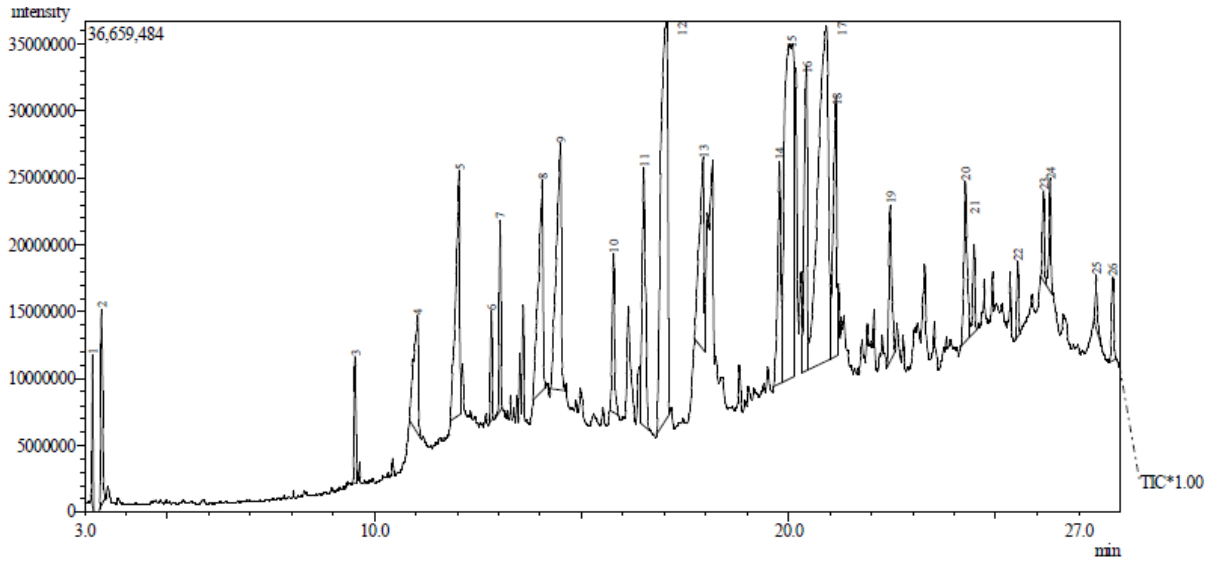

Fig. 1: GC-MS chromatogram of *P. thonningii* leaf extract

Table 1: Phytochemical constituents of leaf extract of *P. thonningii*

Peak no	Components	Retention time(min)	Percentage composition (%)
1	4-Hydroxy-5-methyl-3-propyl-2-hexanone	3.197	0.58
2	3,3-Dimethyl-2-hexanone	3.411	1.62
3	Methyl dodecanoate	9.534	1.05
4	Pentadecanoic acid	11.043	2.62
5	Dodecanoic acid	12.043	4.84
6	Sulfurous acid, butyl cyclohexylmethyl ester	12.821	0.76
7	Methyl tetradecanoate	13.034	1.36
8	1-Pentadecane carboxylic acid	14.049	4.64
9	Octadecanoic acid	14.490	5.76
10	3-Methyl cyclopentane -1,2-diol	15.772	1.87
11	Methyl 11-octadecanoate	16.501	4.20
12	Methyl hexadecanoate	17.063	12.80
13	Heptadecanoic acid	17.936	4.25
14	2-methyl -1-octanol	19.776	3.35
15	Linolelaidic acid, methyl ester	20.098	15.62
16	Methyl n-octadecanoate	20.425	4.10
17	cis-Oleic acid	20.913	16.76
18	Aqua Cera	21.141	4.29
19	Hexadecyl 2,2,333- pentafluoro propanoate	22.451	2.25
20	Undecylenoyl chloride	24.269	2.46
21	Eicogane	24.471	0.90
22	Morpholine, 4-nonanoyl	25.531	0.56
23	Methyl 2-oxohexadecanoate	26.147	0.80
24	Tetracosane	26.312	1.06
25	Heptadecane	27.423	0.57
26	Oleic diethanolamide	27.826	0.93

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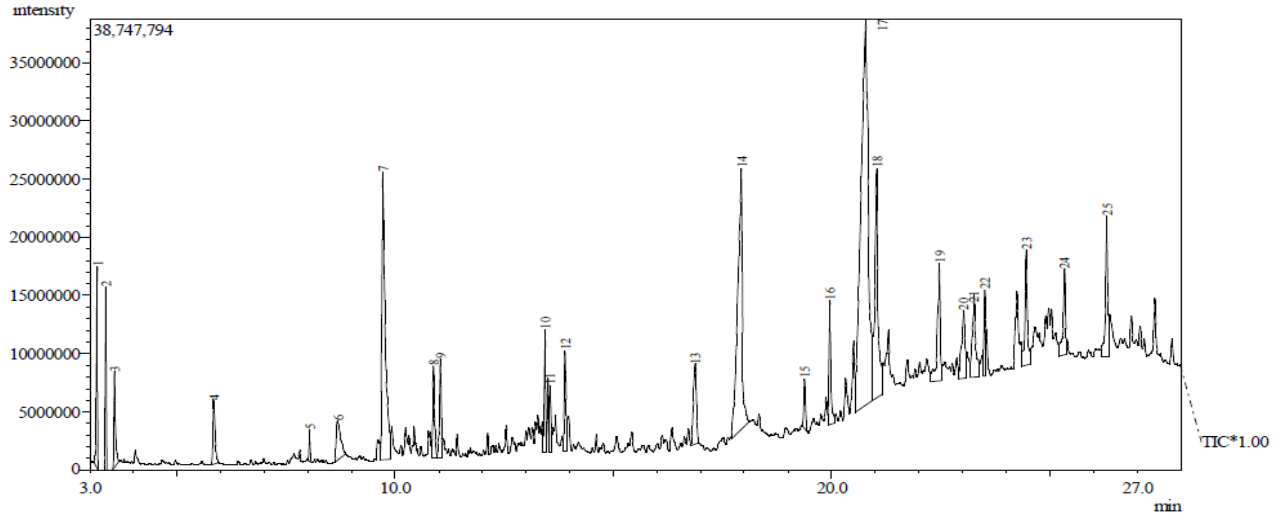

Fig. 2: GC-MS chromatogram of *P. thonningii* flower extract

Table 2: Phytochemical constituents of flower extract of *P. thonningii*

Peak no	Components	Retention time(min)	Percentage composition (%)
1	2-Methyl-2-pentanethiol	3.172	1.87
2	2,2-Dimethyl pentanal	3.372	1.60
3	2-Bromo-4- methylpentane	3.371	1.52
4	Benzeneethanol	5.844	1.48
5	2,4-Dimethyldecane	8.039	0.38
6	p-Vinylguaiacol	8.676	1.78
7	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)	9.716	9.91
8	2,3-Dimethyldodecane	10.877	1.60
9	(Z,E)- alpha-farnesene	11.039	1.72
10	Octadecane	13.434	1.78
11	trans-farnesol	13.554	1.12
12	trans-trans-farnesal	13.895	1.91
13	Hexadecane	16.875	2.45
14	Palmitic acid	17.934	12.65
15	Oxalic acid, isobutyl undecyl ester	19.381	0.90
16	Octadecyl vinyl ether	19.961	2.54
17	Oleic acid	20.782	29.01
18	Stearic acid	21.030	7.66
19	3,8-Dimethylundecane	22.469	3.45
20	Heneicosane	23.026	2.34
21	Oleic acid amide	23.263	3.10
22	4-methyldodecane	23.510	1.31
23	Eicosane	24.457	2.56
24	Tetratetracontane	25.338	2.11
25	11-Decyltetracosane	26.304	3.25

Antibacterial Screening

Table 3: Antibacterial activity of leaf extract of *P. thonningii*

Bacterial strains used	Zone of inhibition (mm)	MIC (%)	Positive standard GT (mm)
E. coli	18.4 ± 0.2	20	24.2 ± 0.3
S. aureus	16.2 ± 0.3	25	23.2 ± 0.2

Notes: Values are in mm and include the diameter of the paper disc (5 mm) and are expressed as the mean of triplicate results ± standard deviation; **MIC**, minimum inhibitory concentration; **GT**, gentamycin.

Table 4: Antibacterial activity of flower extract of *P. thonningii*

Bacterial strains used	Zone of inhibition (mm)	MIC (%)	Positive standard GT (mm)
E. coli	19.3 ± 0.2	20	24.2 ± 0.3
S. aureus	20.2 ± 0.3	20	23.2 ± 0.2

Notes: Values are in mm and include the diameter of the paper disc (5 mm) and are expressed as the mean of triplicate results ± standard deviation; **MIC**, minimum inhibitory concentration; **GT**, gentamycin.

Gas Chromatography / Mass Spectrometry Analysis

GC analysis was carried out in Shimadzu Japan gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm × 50 m) and the conditions were as follows: temperature programming from 80–280 °C held at 80 °C for 1 min., at 200 °C for 4 min. (rate 10 °C/min), and finally at 280 °C for 5 min. (rate 10 °C/min). The injection temperature was 250 °C. GC/MS analysis was conducted using GCMS-QP 2010 Plus (Shimadzu, Japan) with column oven temperature of 80 °C. The carrier gas was helium with a pressure of 108.2 Kpa. All solvents used were of analytical grade and were procured from Merck, Germany. The components of the extract were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature as well as using the database of National Institute of Standards and Technology (NIST) [7].

Antibacterial Activity Screening

The bacteria organisms used for the *in vitro* antibacterial screening were *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The test organisms were clinical isolates of human pathogens obtained from stock cultures at the Federal Medical Centre, Umuahia, Abia State, Nigeria. With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminium foil and sterilised in an autoclave at 121 °C for 15 min. They were however used within 48 h of production. The sensitivity of each test microorganism to the plant extracts was determined using the Disc Diffusion Technique [8]. A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the inoculum was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the extracts bearing paper discs were carefully placed on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 h in an incubator at 37 °C. They were examined daily for growth and for the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimetre rule. Gentamycin was used as a standard. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of the sample having different zones and selecting the lowest concentration.

Statistical Analysis

The antibacterial results were determined in triplicate and were reported as the mean ± standard deviation.

RESULTS AND DISCUSSION

GC/MS Analysis

The GC/MS chromatogram of the leaf extract of *P. thonningii* and the chemical components identified from it are shown in Figure 1 and Table 1 respectively. Twenty-six phytochemical components were identified in the leaf comprising esters (47.23 %), fatty acids (38.87 %), alcohols (5.22 %), hydrocarbons (2.53 %), organic chloride (2.46 %), ketones (2.20 %), amide (0.93 %) and aromatic (0.56 %). As shown in Figure 2 and Table 2, twenty-five phytochemical components were identified in the flower consisting fatty acids (52.42 %), hydrocarbons (32.74 %), terpene/terpenoids (4.75 %), ether (2.54 %), thiol (1.80 %), phenolic (1.78 %), halogenoalkane (1.52 %), aromatic alcohol (1.48 %) and ester (0.90 %).

The major constituents in the leaf were esters (47.23 %) while the major constituents in the flower were fatty acids (52.42 %). Notice that though the esters constituted the highest components in the leaf, they were the least components in the flower. While terpenoids, ether, thiol, phenolic, and halogenoalkane were detected in the flower, they were absent in the leaf. Also, ketones and amide found in the leaf were absent in the flower. These indicate that the phytochemical profiles of these two plant materials, though from the same plant, are not the same. However, oleic acid was the highest compound in both the leaf (16.76 %) and the flower (29.01 %). Kwaji *et al.*, [9] reported the results of the qualitative analysis of the leaves of *P. thonningii*. Here, the volatile components of the leaf and flower have been determined quantitatively. *P. thonningii* finds several applications in traditional herbal medication probably due to the presence of these phytochemicals.

The results of the antibacterial screening of the leaf and flower extracts of *P. thonningii* are shown in Table 3 and Table 4. *E. coli* is a Gram-positive bacterium while *S. aureus* is a Gram-

negative bacterium. The flower extract showed more potent antibacterial activity against the two pathogens than the leaf extract. However, the leaf extract exhibited more inhibition against *E. coli* while the flower extract inhibited *S. aureus* more. MIC is lower in the flower extract than in the leaf extract indicating better efficacy. Gentamycin was used as a standard antibacterial agent and its sensitivity was comparable to those of the extracts. The ability of the extracts to show significant antibacterial activity against the tested organisms could be the reason why the plant is used in herbal medication for the treatment of diseases and infections owing to the presence of enormous bioactive phytochemicals in them. The difference in inhibitory activity might have emanated from the different phytochemical profiles of these plant materials.

CONCLUSION

The phytochemical constituents of the leaf and flower extract of *P. thonningii* have been explored using GC/MS technique. Comparative studies reveal that though the plant materials were obtained from the same plant, their phytochemical constituents are not the same. Hence their antibacterial functions were different. The pronounced antibacterial activity demonstrated by these extracts justifies the use of the plant in traditional herbal medication for the treatment of diseases and infections.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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